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Karyomorphological observations of *Apostasia nuda* and *Neuwiedia veratrifolia* (Apostasioideae, Orchidaceae)

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Apostasia nuda と *Neuwiedia veratrifolia* (ラン科ヤクシマラン亜科) の核形態学的観察

The genera *Apostasia* and *Neuwiedia* possess peculiar and primitive characters among Orchidaceae, i.e., perianths are all petaloids which have not yet differentiated into petals and sepals, two or three stamens separate at the base, placentation is axial, pollen grains are monads, etc. On the basis of the above characters, both genera are considered to belong to the same group and are situated at the most primitive position of Orchidaceae. For examples, Melchior (1964) divided Orchidaceae into two subfamilies, Cypripedioideae and Orchidioideae, and further subdivided the former into two tribes, Apostasieae and Cypripedieae. Cronquist (1981) treated that Orchidaceae consist of three subfamilies, namely, Apostasioideae, Cypripedioideae and Orchidioideae. Thus, Apostasioideae is a very important group for clarification of the phylogeny of the Orchidaceae.

Although much information for the groups of Orchidioideae has been published, these important, key-point plants have not been attempted to observe cytological features except for *Neuwiedia singapureana* (Larsen 1968). The reason why karyological studies were rare is probably that both genera, *Apostasia* and *Neuwiedia*, are rarely distributed at tropical areas and are difficult to cultivate or transplant in gardens. Fortunately, I have had opportunities to approach to these interesting materials at the Sumatra Island during the project of Sumatra Nature Study (SNS). This report describes the karyomorphological characteristics of both *Apostasia nuda* and *Neuwiedia veratrifolia*, and discusses their karyological relationships to Cypripedioideae.

Materials and methods Fresh materials were collected from Bt. (hill) Pinang Pinang, ca. 600 meters altitude from sea level, Ulu Gadut area, about

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20 km east from Padang city, West Sumatra, Indonesia (cf. Hotta 1986). This area is covered with dense canopies of hill forest of height about 40-50 meters. Both species usually grow at the dark forest floor of hill ridges (Kohyama & Hotta 1986). At flower season both species are very conspicuous by bright yellow flowers. It seems that both species extremely restrict the flower season. Once, I observed almost all individuals synchronously bloomed, but at the other time, there was no flower over all.

Chromosome observations of somatic cell division at root tips of *N. veratrifolia* were carried out by the same methods as the previous report (Okada 1986) at SNS laboratory of Ulu Gadut campus, Andalas University, Padang, West Sumatra.

Root tips of *A. nuda* were very hard to squash by conventional techniques, so following methods were employed. Root tips were pretreated by ca. 0.2% colchicine aqueous solution at ca. 25°C for 4 hrs (it was impossible to keep temperature at lower than 20°C in field). They were fixed by a mixture of EtOH : chloroform : acetic acid=2 : 1 : 1, modified Calnoy's fluid, for more than one night, then moved into 70% EtOH and stored. Materials were moved into water through EtOH series. Cell walls were digested by the enzyme treatment (mixture of 2% cellulase (Onozuka RS, Yakult Honsha Co., Ltd.), 0.2% pectolyase (Y-23, Seishin Pharm.) and 0.6 mol mannitol, pH 5.5) at 37°C for 20 min. Macerated root tips were rinsed by water for 10 min, and moved into 45% acetic acid for several min. Meristematic tissues were picked up and stained by 2% aceto-orcein for 40 min. Materials were then squashed.

Voucher specimens are deposited in Kyoto University (KYO).

Results *Apostasia nuda* R. Br. ex Wall.: Somatic chromosome number of this species was counted to be 2n=48 as shown in Figs. 2, 3 and 6. The report of 2n=ca. 60 by Okada (1986) was a miscount due to the difficulty to prepare good preparations for hard cell walls. The morphology of interphase nuclei was that chromatin was stained weakly and granularly (Fig. 1). A few dark stained, round shaped and small pycnotic bodies were observed around nucleolus. At prophase, heterochromatic segments were distinguishable from euchromatic ones (Fig. 2). Heterochromatic segments were very few and distributed at proximal parts of arms. Chromosome sizes at metaphase were very small, about 1 μm, as shown in Figs. 3 and 6. These karyomorphological characteristics correspond to the prochromosome type nucleus (Tanaka 1971).

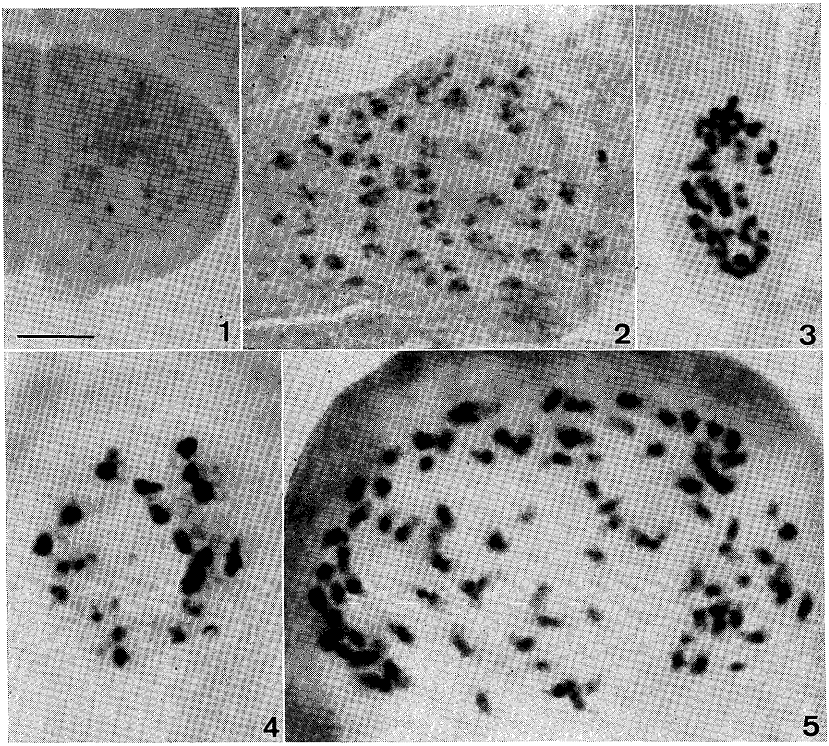

Fig. 1-5. Photomicrographs of somatic chromosomes. 1-3: *Apostasia nuda*, 2n=48. 4-5: *Neuwiedia veratrifolia*, 2n=96. 1, 4: interphase. 2, 5: prophase. 3: metaphase. Bar, 5 μm.

Neuwiedia veratrifolia Bl.: Somatic chromosome number of this species was counted to be 2n=96 (Figs. 5, 7). Interphase nuclei possessed distinctive, large and round shaped heteropycnotic bodies (Fig. 4). The other parts were stained by granulous condition. At prophase, heterochromatic segments were distinguishable from euchromatic ones (Figs. 5, 7). The former was distributed at proximal regions of arms and contacted the latter by clear cut connection. Chromosome size at late prophase ranged from ca. 3 μm to 2 μm. In this study metaphase chromosomes were not observed, but it may be said from the cases of Polycarpicae (Okada 1975) that chromosome sizes at metaphase become less than 2 μm. The karyomorphology of this species was categorized as prochromosome type (Tanaka 1971) as same as the former species.

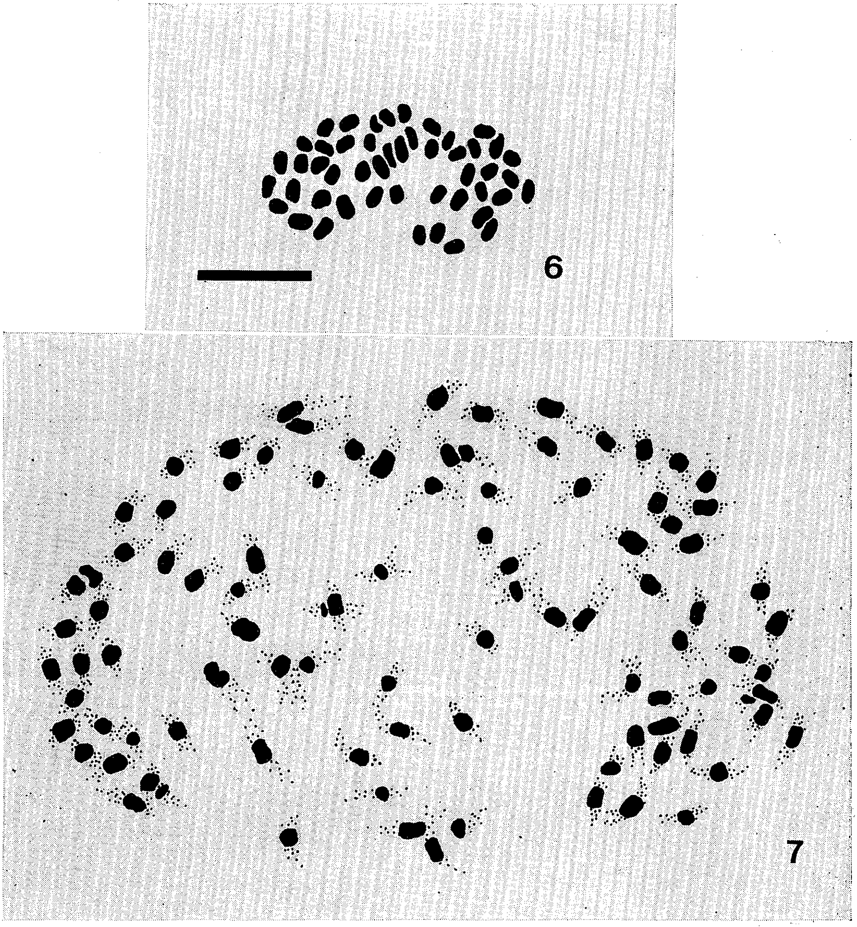

Fig. 6-7. Metaphase chromosomes of *Apostasia nuda*, 2n=48, (6) and prophase chromosomes of *Neuwiedia veratrifolia*, 2n=96, (7). Bar, 5 μm. These sketches, 6 and 7, correspond to Figs. 3 and 5, respectively.

Discussion It is clear that karyomorphological characteristics of *Apostiasa nuda* and *Neuwiedia veratrifolia* are quite distinct from the group of Cypripedioideae (Tab. 1). Both species exhibit a typical nucleus of prochromosome type (Tanaka 1971). Karasawa (1979, 1980) and Karasawa & Aoyama (1986) reported karyomorphology of many species belonging to the genera, *Cypripedium*, Tab. 1. Chromosome characteristics of both subfamilies, Apostasioideae and Cypripedioideae of Orchidaceae (after Cronquist 1981). Symbols of "C", "D" and "P" indicate chromocenter, diffuse and prochromosome type, respectively.

genus	basic number	karyomorphology	chro. size
Apostasioideae			
Apostasia	12	P	ca. 1 μm
Neuwiedia	12	P	<2
Cypripedioideae			
Cypripedium	10	D (C)	26–16
Paphiopedilum	13	C	18– 8
Phragmipedium	9	C	7– 5

Paphiopedilum and *Phragmipedium* of Cypripedioideae. According to their detailed observations, two of three genera, *Paphiopedilum* and *Phragmipedium*, are commonly categorized into complex chromocenter type, while *Cypripedium* dark diffuse type. *C. debile* (subgenus Retiervia) exceptionally exhibits complex chromocenter type. Karasawa & Aoyama (1986) considered the latter species belongs to a different group from the others. Chromosome size may express clearly the differences between both subfamilies (Tab. 1). Both species have very small chromosomes of less than 2 μm, while species belonging to Cypripedioideae possess very large chromosomes, that is 26 to 5 μm.

Basic chromosome number of both genera are considered to be $x=12$ judged from the somatic chromosome numbers. $2n=48$ of *A. nuda* is a tetraploid level of $x=12$, while $2n=96$ of *N. veratrifolia* an octaploid level of $x=12$. Larsen (1968) reported $2n=$ca. 144 for *N. singapureana*, which is probably dodecaploid of $x=12$. This basic chromosome number, $x=12$, is different from those of Cypripedioideae, such as $x=9$ in *Phragmipedium*, 10 in *Cypripedium* and 13 in *Paphiopedilum* (Karasawa 1979, 1980, Karasawa & Aoyama 1986).

Two extreme differences between both subfamilies appear on the change of chromosome numbers. One is that Apostasioideae have common basic number within subfamily, while every genera of Cypripedioideae possess peculiar basic number. Another is that chromosome numbers have changed to higher euploidy in Apostasioideae vs. the aneuploid changes arisen from centric fission have performed in *Paphiopedilum* and *Phragmipedium* at lower ploidy level.

On the basis of both features, karyomorphology and chromosome number, any close karyological relationship between Apostasioideae and Cypripedioideae can not be detected.

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スマトラ島に分布する *Apostasia nuda* と *Neuwiedia veratrifolia* の核形態学的観察を行なった。両属は花被片が未分化，おしべが基部で合着しているだけで上部では離生している，中軸胎座，花粉は単粒であるなど，ラン科の中にあって原始的な形態を多く持ち，ヤクシマラン亜科に入れられ，ラン科の中で最も原始的な群とされている。ラン科植物の核学的研究は相当多く発表されているが，これらヤクシマラン亜科の植物はその分布が熱帯から亜熱帯に限られ，また比較的まれなものであること，移植が難しいことなどのために，今までわずかにタイ産の1種 *Neuwiedia singapureana* で $2n=ca. 144$ と報告されているにすぎない(Larsen 1968)。筆者はスマトラ自然研究 (SNS) 計画に参加して西スマトラに帯在した折にこれらの植物に接する機会があり，核形態を観察することが出来た。そこで本報告では両属の核形態学的特徴を記載し，アツモリソウ亜科との核形態学的関連について議論を試みた。

両種とも共通して前染色体型の核形態を示した。これはアツモリソウ亜科が分散型，または染色中央粒型を示す (Karasawa 1979, 1980, Karasawa & Aoyama 1986) のと比べて明瞭に異なる点である。染色体の大きさの比較はその一側面として両亜科の違いを明瞭にしている。即ち，ヤクシマラン亜科では最大染色体は 2 μm 以下の大きさであるが，アツモリソウ亜科の最大染色体はアツモリソウ属で 26–16 μm, *Paphiopedilum* 属で 18–8 μm, *Phragmipedium* 属で 7–5 μm と相当に大きな違いが見られた。染色体基本数は両属とも共通して $x=12$ と考えられる。*Apostasia nuda* の $2n=48$ はその4倍体，*Neuwiedia veratrifolia* の $2n=96$ はその8倍体，また，*N. singapureana* の $2n=ca. 144$ (Larsen 1968) はその12倍体と考えられる。このように高次の倍数性の見られるのは大変興味深い。アツモリソウ亜科の属はいずれも 2–4 倍体のレベルに留まり，動原体切断による異数性的染色体数変異をしている (Karasawa 1979, 1980, Karasawa & Aoyama 1986) のに比べ，明らかに核学的進化の方向が異なっている。ヤクシマラン亜科に見られる $x=12$ はアツモリソウ亜科の染色体基本数 $x=9$ (*Phragmipedium* 属, Karasawa 1980), 10 (*Cypripedium* 属, Karasawa & Aoyama 1986), 13 (*Paphiopedilum* 属, Karasawa 1979) のいずれとも異なる。以上の様に，核形態の上からも，染色体基本数の上からも，ヤクシマラン亜科とアツモリソウ亜科との間に直接の関連は考えられない。

□豊国秀夫：**日本の高山植物** 719 pp. 1988. 山と渓谷社，東京. ¥4500. まえに紹介した「日本の樹木」「日本の野草」のシリーズである。ところどころにワンポイントリサーチとして，種や属の検索表や小文が挿入されている。 (金井弘夫)